

## **Supplementary Information**

### **Remdesivir Metabolite GS-441524 Effectively Inhibits SARS-CoV-2 Infection in Mouse Models**

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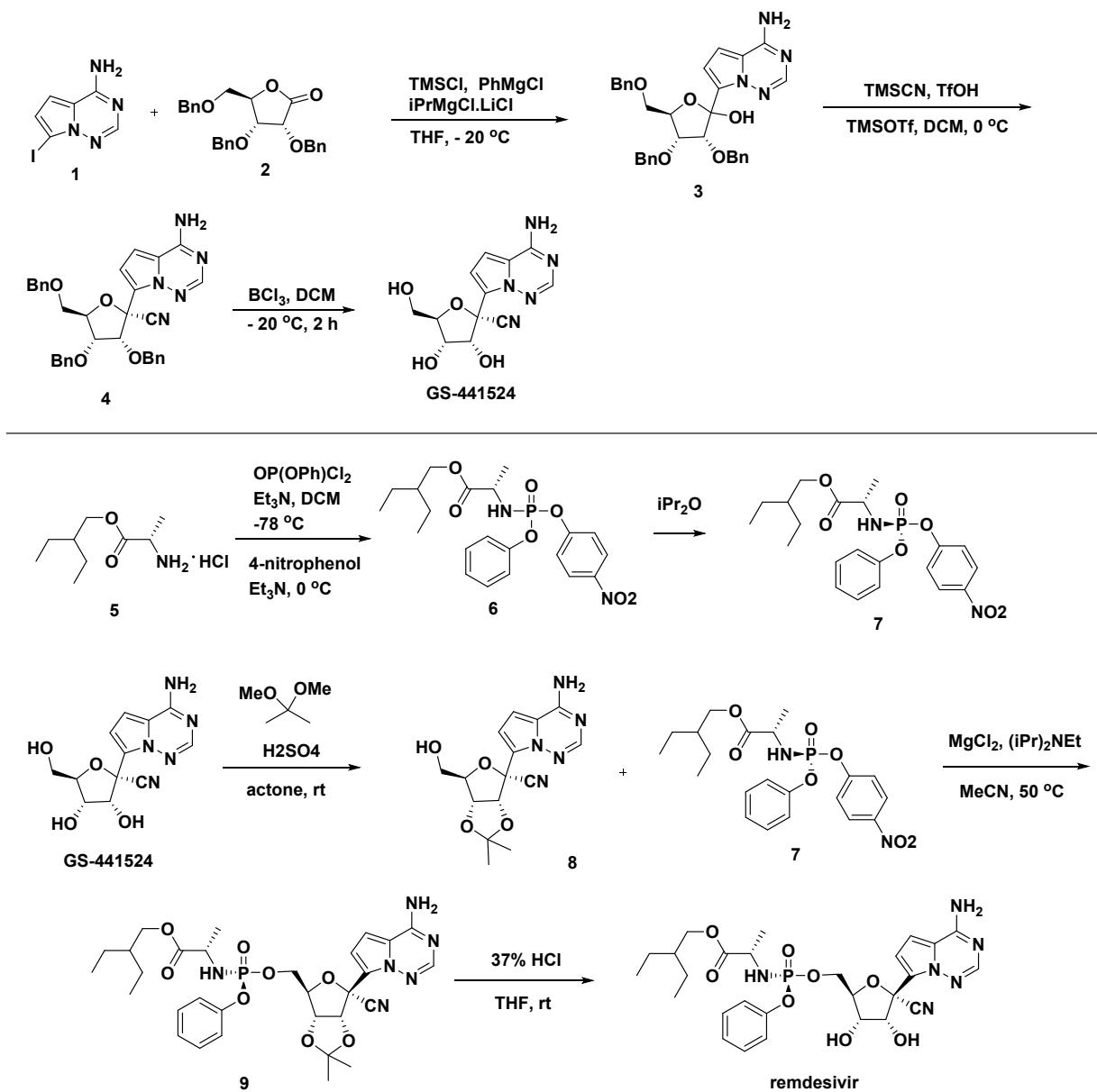
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**Scheme S1.** The comparison of synthesis of remdesivir and GS-441524<sup>1-2</sup>



**Table S1. Primers used in these studies**

Constructs	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Monkey GAPDH	ACATCAAGAAGGTGGTGA AGCAGGC	GTTGAAGTCGGAGGAGACCAC CTGG
Human GAPDH	ATGACATCAAGAAGGTGG TG	CATACCAGGAAATGAGCTTG
mouse GAPDH	AGGTCGGTGTGAACGGAT TTG	TGTAGACCATGTAGTTGAGGTC A
SARS-CoV-2 N	CCAGGTAACAAACCAACC AA	TGAGTGAGAGCGGTGAACCAA
MHV-A59 membrane protein	ATACCTCGGGAACCATGG C	GAGATAAAACCAGTAACAGCG TGC

**Table S2. Pharmacokinetics parameters of GS-441524 over 24h in rat<sup>a</sup>**

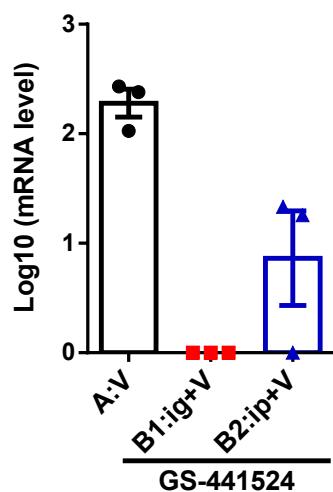
	GS-441524 (iv)	GS-441524 (ig)
AUC(0-24h)( $\mu$ g/L*h)	546002±277019	18642±4346
AUC(0-∞)( $\mu$ g/L*h)	546194±277230	19992±3588
t <sub>1/2</sub> (h) <sup>b</sup>	1.84±0.48	5.64±4.06
T <sub>max</sub> (h)	0.10±0.04	0.94±0.77
C <sub>max</sub> ( $\mu$ g/L)	163616.5±3747.3	2708.0±1308.8
F %		3.41±0.80

<sup>a</sup>each group had four rats; <sup>b</sup>t<sub>1/2</sub> was calculated as the terminal elimination half-life.

**Table S3: PK parameter of remdesivir and its metabolite GS-441524 following single-dose administration of remdesivir within 48h in rat<sup>a</sup>**

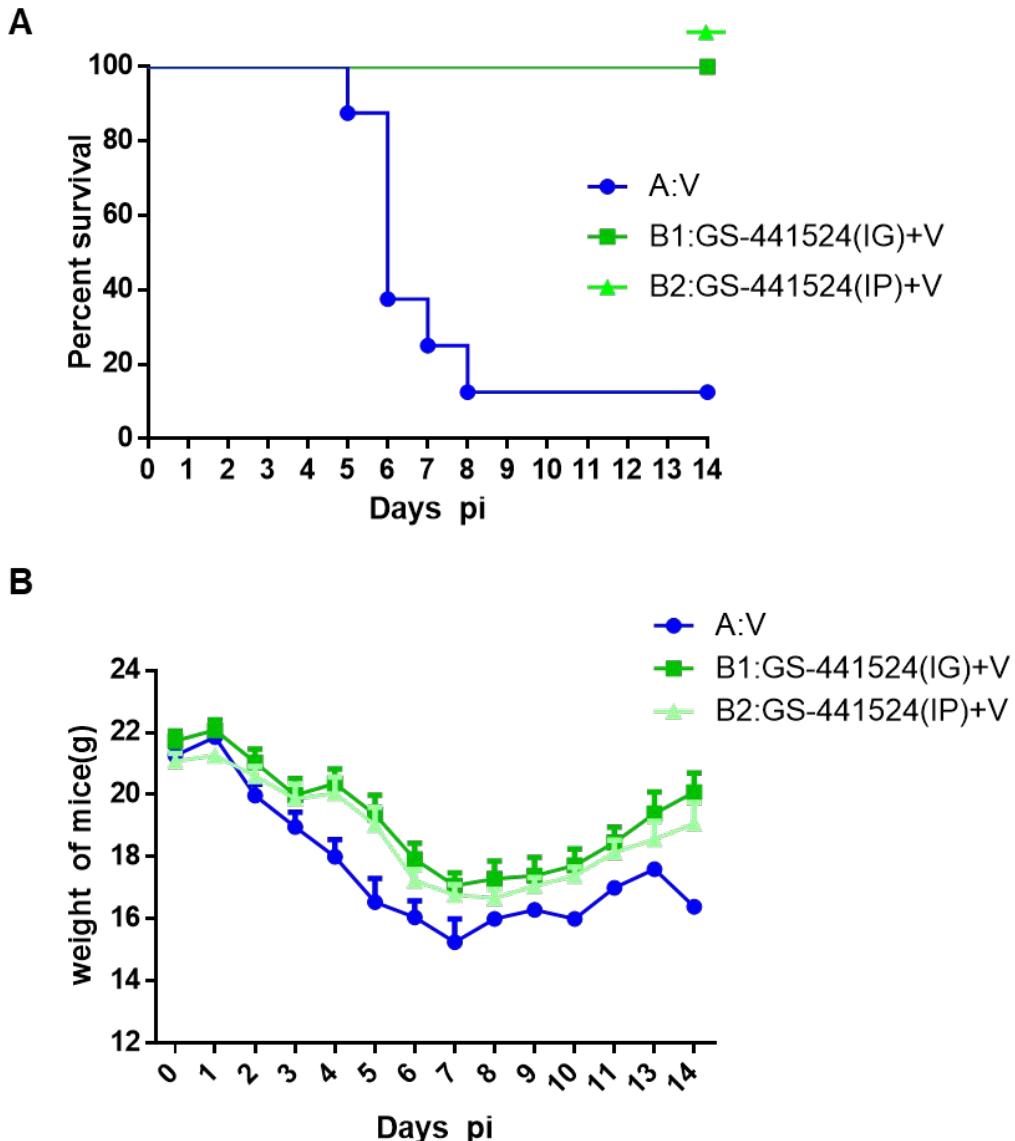
Detected compound	IV <sup>b</sup>		IG <sup>c</sup>	
	remdesivir	GS-441524	remdesivir	GS-441524
AUC(0-t) ( $\mu\text{g/L} \cdot \text{h}$ )	744538.1±1485949.5	510574±187733	1969±1327	15817±1828
AUC(0- $\infty$ ) ( $\mu\text{g/L} \cdot \text{h}$ )	744538.1±1485949.5	545979±181923	1969±1327	16422±2133
$t_{1/2}$ (h)	2.23±1.05	12.83±3.23	3.51±0.16	10.97±2.89
T <sub>max</sub> (h)	12.27±23.82	2.54±3.75	24.25±27.42	1.69±1.73
C <sub>max</sub> ( $\mu\text{g/L}$ )	298.34±203.49	30231.2±14150.7	218.3±209.9	1288.7±486.4
F %		0.26±0.18		3.10±0.36

<sup>a</sup> each group had 4 SD rat; <sup>b</sup> remdesivir was administrated via IV injection at a single dose of 30mg/kg; <sup>c</sup> remdesivir was administrated via IG injection at a single dose of 30mg/kg



**Figure S1. GS-441524 reduce MHV-A59 viral copies in liver at 1 dpi.** Liver tissue of 3 mice in group A, B1 and B2 were harvested and the viruses in liver were analyzed by

qRT-PCR.



**Figure S2. *In vivo* anti-virus efficacy of GS-441524 in mouse MHV model.** The *in vivo* anti-MHV activity of GS-441524 by IG and IP injection was repeated. Mice were randomly divided in 3 groups: Group A: MHV-A59 infected, untreated control; group B1: 100 mg/kg GS-441524 IG 0.5 hours post infection (hpi), then 50 mg/kg daily in infected mice; Group B2: 100 mg/kg GS-441524 IP 0.5 hpi, then 50 mg/kg daily in infected mice. Note: V=virus. Survival curves of mice in Groups A (blue) B1 (dark green) and B2 (light green). Note: N=8 per group. (A) Body weights of animals in the 3 groups; Note: N=8 per group. (B)

The MHV infection caused 7 mice dead (87.5%) and G-441524 treatment mice displayed a survival rate of 100% at the end of the experiment at 14 dpi.

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